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(54) Title: FEED ADDITIVES AGAINST DISEASSE INFECTION IN TERRESTRIAL AND AQUATIC ANIMALS

(57) Abstract: An animal feed additive, for aquatic or terrestrial animals, which has a primary composition and a secondary composition, both of which maintain their bioactivity during feed processing and storage. The compounds can be volatile, or non-volatile. A method for feeding an aquatic or terrestrial animal a feed, which includes providing the animal with a feed additive with a primary and secondary composition that maintain their bioactivity. The feed, and the method of feeding, protect the animal from disease.

TITLE OF THE INVENTION

[0001] Feed Additives Against Disease Infection in Terrestrial and Aquatic Animals

BACKGROUND OF THE INVENTION

[0002] Losses due to disease in world agriculture are estimated to be in the billions of

5 dollars annually. The recent emergence of pathogens resistant to conventional anti-microbial agents has imposed an additional burden leading to an intensified search for new strategies for the prevention and control of infectious diseases. Whereas antibiotics are often administered to treat outbreaks of disease, a number of factors in their use are known to be problematic, such as solubility, palatability, cost, delivery, government restrictions, 10 and the small differential between therapeutic and toxic levels (Dixon 1994). As a result, only a few antibacterial agents are currently available for use in agriculture, especially for food animals. The use of chemotherapeutic agents is becoming even more limited because of growing concerns for consumer health and the accumulation of substances in the environment (Anderson 1992). An additional problem is that continual use of antibiotics 15 results in increasing resistance in the target micro-organisms (Solomon, Berg et al. 1993). Increasing resistance of bacterial pathogens to antibacterial agents is being reported in agricultural studies worldwide (Dixon 1994).

[0003] The efficacy of treating infectious diseases with various plant extracts is well known, and is increasingly recognized as a desirable alternative to synthetic drugs. For

20 example, U.S. Pat. No. 4,886,665 teaches the use of a pharmaceutical preparation of oats and nettle extracts. U.S. Pat. No. 4,671,959 discloses the use of mixtures of natural oils for stress reduction. U.S. Pat. No. 5,178,865 discloses a plant extract mix, which inhibits infection of human immunodeficiency virus (HIV) in vitro.

[0004] One of the most powerful groups of bioactive compounds is the polyphenolics,

25 which represents a diverse group of compounds (Heim, Tagliaferro et al. 2002). Polyphenolics widely occur in a variety of plants, some of which enter into the food chain. Although some polyphenols are considered to be non-nutritive, interest in these compounds has arisen because of their possible beneficial effects on health.

[0005] For instance, quercetin (a flavonoid) has demonstrated anti-carcinogenic activity

30 in experimental animals (Deschner, Ruperto et al. 1991). Epigallocatechin gallate has been reported to be the pharmacologically active material in green tea that inhibits mouse skin

tumors (Okuda, Yoshida et al. 1995). Ellagic acid has also been shown to possess anti-carcinogenic activity in various animal tumor models (Boukharta, Jalbert et al. 1992).

[0006] U.S. Pat. No. 5,990,178 and WO 96/37210 disclose pharmaceutical compositions for treating a disease in poultry induced by hemoflagellates. Polyphenols derived from green tea have been reported to significantly decrease the amount of Clostridium perfringens and other Clostridium spp. (putrefactive bacteria) and significantly increase the amount of Bifidobacterium spp. (acid forming bacteria) in animals (Ishihara, Chu et al. 2001). Hara and colleagues determined that green tea polyphenols had bactericidal effects, and that polyphenolics present in tea include gallicatechin and epicatechin gallate (Hara, Orita et al. 1995).

[0007] One compound that has received particular attention from the research community is the polyphenolic glucoside oleuropein, which is extracted from the leaves or fruits of the olive tree. A number of scientific studies have shown this compound to have anti-viral, anti-fungal, anti-bacterial (Koutsoumanis; el al., 1998; Aziz, et al., 1998; Tranter, et al., 1993; Tassou, et al., 1995), anti-oxidant (de la Puerta, et al., 1999; Vissioli, 1998a), as well as, anti-inflammatory properties (Visioli, et al., 1998b).

[0008] It is believed that the antimicrobial activity of these organic phenolic compounds is due to the destruction of lipids in the microorganism's outer membrane. The antimicrobial action of polyphenolics is related to their ability to denature proteins, and they are generally classified as surface-active agents. They act by causing leakage of cytoplasmic constituents, such as proteins, potassium glutamate, and phosphate from bacterial cells. This cytoplasmic leakage may be due to disruption of the peptidoglycan cell wall or direct damage to the plasma membrane.

[0009] Furthermore, it is believed that the efficacy of the antimicrobial compound will not be compromised due to the development of pathogen resistance. The British Pharmacopoeia (1996 Edition) reports that microorganisms do not build resistance to benzyl alcohol, phenols, polyphenols, and similar products.

[0010] Another major group of compounds that also possesses biological activity is plant volatiles or essential oils. A number of plant extracts and pure isolates have been mentioned as containing substances that interfere with or inhibit infection by viruses. Some of these compounds, like galangin, when used in concentrations ranging from 12-47

micrograms per milliliter, showed significant antiviral activity against herpes simplex virus -1 (HSV-1) and Cox B1 (Meger, Afoloyan et al. 1997).

5 [0011] The plant *Hyptianthera stricta* L. is used against encephalitis-causing viruses providing 75% inhibition at 62.5 micrograms per milliliter (Saxena, Gupta et al. 1997). The essential oil of *Melaleuca alternifolia*, at concentrations of 100, 250, and 500 ppm, was found to be effective in decreasing local lesions of tobacco mosaic virus (TMV) on the host plant *Nicotiana glutinosa* (Bishop 1995).

10 [0012] The essential oil of *Salvia fruticosa* and its isolated components (thujone and 1,8 cineole) exhibited activity against eight bacterial strains and high levels of antiviral activity against HSV-1 (Sivropou, Nikolaou et al. 1997).

[0013] Terpenoids (citral, geraniol, eugenol, menthol, cinnamic aldehyde) have also been found to inhibit growth of bacteria and fungi and some internal and external parasites (Hooser, Beasley et al. 1986).

15 [0014] Geraniol was found to inhibit growth of *Candida albicans* and *Saccharomyces cerevisiae* strains by enhancing the rate of potassium leakage thereby disrupting membrane fluidity (Bard, Albrecht et al. 1988).

[0015] B-ionone has antifungal activity, which was determined by inhibition of spore germination and growth inhibition in agar (Mikhlin et al, 1983 and Salt et al, 1986).

20 Teprenone has an antibacterial effect on *Helicobacter pylori* (Ishii, 1993). Solutions of eleven different terpenes were effective in inhibiting the growth of pathogenic bacteria in vitro at between 100 ppm and 1000 ppm (Kim, Marshall et al. 1995).

25 [0016] Another microbiocidal family of compounds is the quinones. Quinones are a large and varied group of natural products found in all major groups of organisms. Quinones are a group of aromatic dioxo compounds derived from benzene or multiple-ring hydrocarbons, such as naphthalene and anthracene. They are classified as benzoquinones, naphthoquinones, anthraquinones, and etc., on the basis of the ring system. Quinones with long isoprenoid side chains, such as plastoquinone, ubiquinone and phytoquinone, are involved in the basic life processes of photosynthesis and respiration.

30 [0017] A subset of quinones, designated lapachones, has been shown to have activity against neoplastic cells, as described in U.S. Pat. Nos. 5,969,163, 5,824,700, and 5,763,625. Antiviral activity is mentioned in U.S. Pat. Nos. 5,641,773 and 4,898,870, while antifungal and trypanosidal activity of beta-lapachone is mentioned in U.S. Pat. Nos. 5,985,331,

5,912,241 and 6,482,943. Lapacho is a naturally occurring quinone derived from a tree native to Brazil, *Tabebuia avellaneda*. The major active components are 16 quinones and lapachol; the latter has been used in anti-tumor treatment both orally and parenterally and has exhibited anti-parasitic and anti-inflammatory activity. Another antiviral derivative is
5 beta-lapachone, a simple non-water soluble orthonaphthoquinone, that can be obtained by simple sulfuric acid treatment of the lapachol, or is easily synthesized from seeds of lomatia (Li, Averboukh et al. 1993). Beta-lapachone has been shown to have a variety of pharmacological effects. Numerous derivatives have been synthesized and tested as anti-viral and anti-parasitic agents. Beta-lapachone significantly prolongs the survival of mice
10 infected with Rauscher leukemia virus, probably through inhibition of reverse transcriptase (Schaffner-Sabba, Schmidt-Ruppin et al. 1984; Li, Zhang et al. 1993).

[0018] Although the use of plant-derived compounds is now widely accepted in humans, their use in animals is not yet established. Current disease control tends to rely on treatments using synthetic or chemically manufactured drugs or a purified bioactive extract
15 of plant, rather than using whole plants for treatment, thereby losing minerals, vitamins, glycosides, oils, alkaloids, bioflavonoids, and other substances, in addition to other active compounds present in a plant. These additional substances can provide a synergistic effect, which is absent when purified or synthetic active compounds are used alone. Additionally, the toxicity of purified active compounds is generally higher than when the active
20 compounds are present with the other plant substances. There is a pressing desire for non-antibiotic treatments against animal diseases, which is the focus of this invention.

SUMMARY OF THE INVENTION

[0019] This invention aids in fulfilling these needs in the art. This invention provides a composition of plant-derived bioactive feed additives and a method of use of these additives
25 for the protection or prevention of disease in terrestrial and aquatic animals by adding these additives to their feed, or using them as a specialty treatment feed.

[0020] This invention also provides a method for the use of plant-derived bioactive feed additives either directly or by incorporation into feeds.

[0021] In addition, this invention provides a method for protecting animals against
30 disease using plant-derived bioactive feed additives either directly or by incorporating them into feeds.

[0022] In one embodiment, the invention provides a feed additive comprising a primary composition containing a non-volatile bioactive material and a secondary composition comprising a volatile bioactive material. The compositions are mixed together to provide a product.

5 [0023] In another embodiment, the invention provides a feed additive comprising a primary composition containing a non-volatile bioactive material and a secondary composition comprising a stabilized volatile bioactive material. This additive provides enhanced protection from disease relative to either composition alone.

10 [0024] In another embodiment, the invention provides a feed additive comprising a primary composition containing a bioactive material and a secondary composition comprising a different bioactive material, wherein the secondary composition is present in 40% or less of the amount of the primary composition.

BRIEF DESCRIPTION OF THE DRAWING

15 [0025] This invention will be described in detail with reference to the drawing, in which:

[0026] Figure 1 presents the bacterial control in Artemia enrichment medium achieved by using the antibacterial composition described in Example 9. A significant improvement in the survival rate of the Artemia was found in enrichments where the antibacterial 20 composition was present versus a control where there was no antibacterial treatment (72 vs. 57% survival in treated versus non-treated, respectively). The top plates show the bacterial growth on bacterial growth medium 16 hours post treatment. The bottom plates show liquid culture from the Artemia enrichment.

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DETAILED DESCRIPTION OF THE INVENTION

[0027] Definitions

[0028] In describing the present invention, the following terminology is used in accordance with the definitions set out below.

30 [0029] Herein, "microbicidal activity" is activity capable of destroying, killing, or inactivating microorganisms. These activities include but are not limited to bactericidal, fungicidal, protozoacidal, and other disinfective activities. A "microbicidal agent" is a compound or composition capable of microbicidal activity.

[0030] The term "food additive" refers to any substance intended to or which may reasonably be expected to result - directly or indirectly - in its becoming a component or otherwise affecting the characteristics of any food. It includes any substance used in the production, processing, treatment, packaging, transportation or storage of food products. It includes bioactive substances that are present at relatively low ratios with the major feed components. It includes bioactive compositions that are added to a complete diet or added separately as tablets, pellets or beads to be consumed directly. Food additives are not meant to fulfill the nutritional needs of the animal but provide some specific benefit.

[0031] "Primary compositions" are materials comprising bioactive compounds, such as,

but not limited to, polyphenols, phenols, organic acids and non-volatile oils or extracts containing flavonoids and isoflavones that are included in the diets of the instant invention. Alternatively, the bioactive materials could be volatile compounds that have been processed to stabilize the volatile compound or entrap the volatile compound so that it is retained in the composition. Such a volatile compound includes, but is not limited to, alcohols, aldehydes, acetates, acetals, terpenes, quinones, and oils or extracts thereof. Such compositions can be made from biomass, single cells, crude preparations (natural or synthetic), and or extracts thereof.

[0032] "Secondary compositions" can be any of the bioactive materials listed in the "primary composition" definition above. However, the secondary compositions are always present, and present at an amount equal to or less than 40% of the primary composition. Such secondary compositions function in concert with the primary compositions for the purposes of this invention in the delivery of enhanced bioactivity.

[0033] "Volatile compounds" are compounds that evaporate or vaporize at normal temperatures and pressures.

[0034] As used herein, "polyphenols" are molecules with two or more phenol moieties.

[0035] Bioactive Compositions

[0036] The compositions according to the instant invention are effective against infectious diseases and infectious disease syndromes, in particular, against parasites, fungi, bacteria (Gram-positive, Gram-negative and, especially, *Vibrio* species) and viral infection. Also, the compositions according to the invention can prevent, ameliorate, or cure the effects of viruses and microbial toxins, such as aflatoxins and enterotoxins.

[0037] Terrestrial animals are subject to a variety of infectious agents, infectious diseases, and disease syndromes with viral, bacterial, protozoal, rickettsial, parasitic and fungal etiologies that include, but are not limited to:

[0038] Bovine herpes virus, which can cause infectious bovine rhinotracheitis, and

5 infectious pustular vulvovaginitis; Bovine virus diarrhea virus (BVD); Campylobacter fetus, which can cause vibriosis; Leptospira spp., which can cause leptospirosis; Brucella abortus, which can cause brucellosis; Haemophilus somnus, which can cause somnus; Neospora caninum, which can cause neosporosis; Tritrichomonas foetus, which can cause Trichomoniasis; Cryptosporidium parvum, Adenovirus, gallisepticum, and Mycoplasma.

10 The bioactive compositions of the invention can prevent, ameliorate, or cure the diseases and syndromes caused by these agents.

[0039] Terrestrial animals are also subject to the infectious diseases, and disease syndromes salmonellosis, Avian tuberculosis (AT), listeriosis, paratyphoid, pasturellosis, streptococcosis, yersiniosis, systemic mycoses, e.g. histoplasmosis and cryptococcosis,

15 aspergillosis, candidiasis, coccidioidosis, sarcosporidiosis, toxoplasmosis, chlamydiosis, Q fever, meningitis, taeniasis, schistosomiasis, mastitis, Udder Edema, Caseous Lymphadenitis, including Abscesses, Contagious Ecthyma, or Sore Mouth, Enterotoxaemia, or Overeating Disease, Foot Rot, Ring Worm, Aspergillosis, Colibacillosis, Encephalomyelitis, Influenza, Tuberculosis, Blackhead, Bordatelloasis, Botulism, Cage

20 Layer Fatigue, Candidiasis, Chicken Anemia Syndrome, Coryza, Cryptosporidiosis, Eastern Equine Encephalitis, Erysipelas, Fowl Cholera, Fowl Pox, Gangrenous Dermatitis, Hemorrhagic Syndrome, Infectious Bronchitis, Infectious Bursal Disease, Infectious Synovitis, Laryngotracheitis, Lymphoid Leukosis, Marek's Disease, Newcastle Disease, Trichomoniasis, West Nile Virus, and Viral Arthritis. The bioactive compositions of the

25 invention can also prevent, ameliorate, or cure these diseases and syndromes.

[0040] Aquatic infectious diseases include, but are not limited to, Bonamiosis, Epizootic Haematopoietic Necrosis, Haplosporidiosis, Infectious Haematopoietic Necrosis, Marteiliosis, Mikrocytosis, Oncorhynchus Masou Virus Disease, Perkinsosis, Spring Viremia of Carp, Taura Syndrome, Viral Haemorrhagic Septicaemia, White Spot Disease,

30 Yellowhead Disease, Bacterial Kidney Disease, which can be caused by *Renibacterium salmoninarum*, Baculoviral Midgut Gland Necrosis, Channel Catfish Virus Disease, Crayfish Plague, Enteric Septicaemia of Catfish, Edwardsiellosis, Epizootic Ulcerative

Syndrome, Gyrodactylosis of Atlantic Salmon, which can be caused by Gyrodactylus salaris, Infectious Hypodermal and Haematopoietic Necrosis, Infectious Pancreatic Necrosis, Infectious Salmon Anemia, Nuclear Polyhedrosis Baculoviroses, Piscirickettsiosis, Red Sea Bream Iridoviral Disease, Spawner-isolated Mortality Syndrome, 5 Viral Encephalopathy and Retinopathy, and White Sturgeon Iridoviral Disease. The bioactive compositions of the invention can prevent, ameliorate, or cure these diseases and syndromes.

[0041] This invention also provides a method of delivering a mixture of bioactive primary and secondary compositions, including volatile compounds, to animals through 10 their feed. The bioactive compounds for use in the compositions can be made synthetically by known methods available commercially, or obtained from the plant or plant part by conventional separation techniques. Plant extracts can be obtained by providing trees, herbs, spices and the like as raw materials and subjecting their wood chips, barks, roots, stalks, leaves, fruits, flowers or the like to extraction, steam distillation, pressing, or the like 15 in a manner known in the art and can be used as they are, without any further processing or treatment. Concerning these source plants, no particular limitation is imposed on their place of origin, weather, harvesting method, handling method, and the like. Stabilization by addition of antioxidants or preservatives to these crude preparations is also contemplated.

[0042] In one aspect, this invention further increases the bioavailability and absorption 20 through the gut wall into the bloodstream of each treatment composition, by mixing several groups of bioactive compounds. Examples are the combinations of phenols or polyphenols (the primary composition) with essential oils, terpenoids or other alcohols (the secondary composition). The secondary composition is added at 40% or less of the amount of primary composition, and is meant to enhance the effectiveness of the feed additive in delivery of a 25 health benefit.

[0043] In the primary or secondary compositions according to the invention, if the 30 bioactive compound is not volatile, it is chosen from the group consisting of polyphenols, flavonoids, isoflavones, curcuminoids, alone, or as a mixture. Preferred embodiments of the primary composition according to the invention include one or more plant bioactive compounds selected from one or more of the following groups:

[0044] a) Phenols, such as thymol, methyleugenol, acetyleneugenol, safrol, eugenol, isoeugenol, anethole, phenol, methylchavicol (estragol; 3-4-methoxyphenyl- 1-propene),

carvacrol, alpha-bisabolol, fornesol, anisole (methoxybenzene), and propenylguaethol (5-propenyl-2-ethoxyphenol);

[0045] b) Polyphenols, such as flavonoids, including tannins, aromadendrines, anthocyanins, catecholins, catechins, oleuropein, and taxifolins;

5 [0046] c) Plant oils or extracts having a high content of phenols from plants such as origanum, thyme, rosemary, orange, clove, fennel, camphor, mandarin, anise, cascarilla, estragon, and pimento;

[0047] d) Oils or extracts having a high content of polyphenols such as olive tree (leaf or fruit extracts), green tea, coffee and cocoa, flavonoids and isoflavones, such as those present in soya, green tea, onions or grapes and curcuminoids, such as are present in ginger; and

10 [0048] e) Acids and/or their physiologically acceptable salts, such as acetic acid, aconitic acid, adipic acid, formic acid, malic acid (1-hydroxysuccinic acid), capronic acid, hydrocinnamic acid (3-phenyl-1-propionic acid), pelargonic acid (nonanoic acid), lactic acid (2-hydroxypropionic acid), phenoxyacetic acid (glycolic acid phenyl ether), phenylacetic acid (alpha-toluenic acid), valeric acid (pentanoic acid), iso-valeric acid (3-methylbutyric acid), cinnamic acid (3-phenylpropenoic acid), citric acid, mandelic acid (hydroxyphenylacetic acid), tartaric acid (2,3-dihydroxybutanedioic acid; 2,3-dihydroxysuccinic acid), fumaric acid, and tannic acid.

15 [0049] In the primary or secondary compositions according to the invention, if the bioactive compound is volatile, it is obtained from the group consisting of alcohols, aldehydes, acetates, acetals, esters and terpenes, and quinines, alone or as a mixture. Such materials can be chosen from the following groups:

20 [0050] a) Alcohols, such as acetoin (acetyl methyl carbinol), ethyl alcohol (ethanol), propyl alcohol (1-propanol), iso-propyl alcohol (2-propanol, isopropanol), propylene glycol, glycerol, benzyl alcohol, n-butyl alcohol (n-propyl carbinol), iso-butyl alcohol (2-methyl-1-propanol), hexyl alcohol (hexanol), L-menthol, octyl alcohol (n-octanol), cinnamyl alcohol (3-phenyl-2-propene-1-ol), alpha-methylbenzyl alcohol (1-phenylethanol), heptyl alcohol (heptanol), n-amyl alcohol (1-pentanol), iso-amyl alcohol (3-methyl-1-butanol), anisic alcohol (4-methoxybenzyl alcohol, p-anisic alcohol), citronellol, n-decyl alcohol (n-decanol), geraniol, beta-gamma-hexenol (3-hexenol), lauryl alcohol (dodecanol), linalool, nerolidol, nonadieneol (2,6-nonadiene-1-ol), nonyl alcohol (nonanol-1), rhodinol, terpineol,

borneol, clineol (eucalyptol), anisole, cumanyl alcohol (cuminol), and 10-undecene-1-ol 1-hexadecanol;

[0051] b) Aldehydes, such as acetaldehyde, anisic aldehyde, benzaldehyde, iso-butyl aldehyde (methyl-1-propanal), citral, citronellal, n-capraldehyde (n-decanal), ethylvanillin, furfural, heliotropin (piperonal), heptyl aldehyde (heptanal), hexyl aldehyde (hexanal), 2-hexenal (beta-propylacrolein), hydrocinnamic aldehyde (3-phenyl-1-propanal), lauryl aldehyde (dodecanal), nonyl aldehyde (n-nonanal), octyl aldehyde (n-octanal), phenylacetaldehyde (1-oxo-2-phenylethane), propionaldehyde (propanal), vanillin, cinnamic aldehyde (3-phenylpropenal), perillaldehyde, and cuminaldehyde;

10 [0052] d) Acetates, such as iso-amyl acetate (3-methyl-1-butyl acetate), benzyl acetate, benzylphenyl acetate, n-butyl acetate, cinnamyl acetate (3-phenylpropenyl acetate), citronellyl acetate, ethyl acetate (acetic ester), eugenol acetate (acetyleugenol), geranyl acetate, hexyl acetate (hexanyl ethanoate), hydrocinnamyl acetate (3-phenylpropyl acetate), linalyl acetate, octyl acetate, phenylethyl acetate, terpinyl acetate, triacetin (glyceryl triacetate), potassium acetate, sodium acetate, sodium diacetate, and calcium acetate;

15 [0053] e) Acetals, such as acetal, acetaldehyde dibutyl acetal, acetaldehyde dipropyl acetal, acetaldehyde phenethyl propyl acetal, cinnamic aldehyde ethylene glycol acetal, decanal dimethyl acetal, heptanal dimethyl acetal, heptanal glycetyl acetal, and benzaldehyde propylene glycol acetal;

20 [0054] f) Esters, such as allicin;

[0055] g) Terpenes, such as camphor, limonene, and beta-caryophyllene;

[0056] h) Quinones, such as benzoquinones, naphthoquinones, anthraquinones, plastoquinone, ubiquinone, phytoquinone, lapacho, lapachones, and beta lapachone; and

25 [0057] i) Bioactive agents and/or alcoholic or glycolic extracts obtained from the plants listed below --

[0058] 1) Oils or extracts having a high content of alcohols, for example, oils or extracts of melissa, coriander, cardamon, and eucalyptus;

[0059] 2) Oils or extracts having a high content of aldehydes, such as oils or extracts of eucalyptus citriodora, cinnamon, lemon, lemon grass, melissa, citronella, lime, and orange;

30 [0060] 3) Oils or extracts having a high content of acetates, such as lavender oil or extract;

[0061] 4) Oils or extracts having a high content of esters, such as mustard, onion, and garlic oils and extracts; and

[0062] 5) Oils or extracts having a high content of terpenes, such as oils and extracts of pepper, bitter orange, caraway, dill, lemon, peppermint, and nutmeg apple.

5 [0063] Extracts of the natural products that will be included in both the primary and secondary compositions have demonstrated antimicrobial activity, antiseptic activity, anti-inflammatory activity, antioxidant activity, enzyme stimulation or inhibition, pigmentation enhancement or control, photoprotective activity, and many other physiological benefits.

10 [0064] The compositions according to the invention do not result in disadvantages in taste, smell, or color of the treated terrestrial or aquatic animal as a food product for human consumption. Therefore, a particular advantage of the compositions described is that they are reliable disease control additives. On one hand, they are effective against a broad range of pathogens, including Gram-positive and Gram-negative bacteria, fungi, and viruses. While on the other hand, they pose no danger to the end consumer of the terrestrial or 15 aquatic animals as food, since it is absolutely harmless towards him and has no microbicidal after-effect in the food.

20 [0065] The microbicidal activity of the compound is effective when the concentration of the compound in the terrestrial or aquatic feed is from 0.01 to 100 grams per kilogram, preferably from 0.05 to 20 grams per kilogram, and more preferably from 0.05 to 10 grams per kilogram.

25 [0066] The activity of the composition employed is independent of the pH and can be used irrespective of feed moisture, fat, protein, and carbohydrate contents. Finally, the compositions of the invention are insensitive to temperature variations within a range of from below zero temperature to over 100 degrees Celsius (i.e., they are both cold and heat resistant).

[0067] Lipophilic plant extracts in liquid form, such as essential oil extracts, can be directly mixed with fish oil without a solvent due to their lipophilic character and spray-coated on the feed prior to consumption.

30 [0068] Dry powdered plant extracts, such as olive leaf or grape seed extracts, can be directly mixed with feed ingredients and pelleted or extruded according to methods known in the art.

[0069] This invention also provides a method to combine plant extracts in dry powder form with liquid form (aqueous or oil), in a mixture suitable for animal consumption.

[0070] It has not been possible to date to combine a powdered extract of one particular bioactive agent with an aqueous or with a lipid or other volatile agent into a single system
5 without losing bioactivity and effectiveness of volatile compounds during feed processing and storage.

[0071] The use of phospholipids as surface-active agents and special processing conditions to form microparticulate compositions is disclosed herein. Surface active agents permit the mixing of a hydrophilic phase and a hydrophobic phase by lowering the surface
10 tension between the two phases, thereby creating micelle structures which, when mixed with a suitable processing procedure, produce stable systems.

[0072] Specifically, this invention discloses a method in which a powdered, volatile, or liquid extract is mixed with a powdered lecithin and complexed with cornstarch containing 50% amylose and attractants. This starch, which is in beadlet form, is stable at high
15 temperatures and in the acidic environment of the digestive tract of the animal. Such a resistant starch-based system increases the bioavailability and absorption through the gut wall into the bloodstream, thereby delivering the bioactive compounds in their active form and at effective doses. Attractants, such as, but not limited to, fish, krill, shrimp, or crab meal hydrolysates, or any combination of aquatic animal meal hydrolysates containing 3-
20 30% w/w, can be dissolved into the slurry, as can 1-5% w/w Betaine and Glycine + Alanine mixture. The addition of protein or polypeptide to the beadlets provides sites and pores that can be opened by digestive tract proteases or other enzymes, which allows the animal to partially digest the composed material. Other essential nutrients or enzymes such as, but not limited to, essential amino and fatty acids, sugars, polysaccharides, minerals, vitamins,
25 proteases, lipases and amylases can be added to the slurry. Finally, 1% (w/v) alginic acid is dissolved in the solution. The solution is then atomized or dropped into a 5% calcium chloride bath to cross-link the alginate polymer. The microparticulate or droplet composition is harvested and can be kept moist, or freeze-dried for later addition in feed formulation, or directly fed to the animal.

[0073] Examples

[0074] Certain embodiments of the invention will now be described in more detail through the following examples. The examples are intended solely to aid in more fully describing selected embodiments of the invention and should not be considered to limit the 5 scope of the invention in any way.

[0075] Example 1 -- Production of microparticles containing the microbicidal composition

[0076] High amylose starch (50% amylose, Hylon V, National Starch and Chemical, 10 Bridgewater, NJ) was dissolved in 1N sodium hydroxide at 50 degrees Celsius. Powdered egg lecithin (Archer-Daniels-Midland Co., Decatur, IL) was added to the alkali slurry and allowed to complex with the starch for 30 minutes. The alkali complex slurry was then neutralized to pH 7.5 with hydrochloric acid. Alginic acid was then dissolved into the slurry and allowed to cool down to room temperature. The primary and secondary compositions 15 were added to the cooled and neutralized slurry as well as 2% w/v of attractants (Aqua Savor, Bentoli, Inc., Homestead, FL). The slurry was then atomized or dropped into 5% w/w calcium chloride plus 1% w/w sodium chloride bath to form microparticles or droplets in a size range between 10 micrometers and 5000 micrometers. The particles were washed with tap water on a fine mesh screen and kept refrigerated at 4 degrees Celsius until use.

20 The composition of the particulate material is provided in Table 1.

Table 1. Microparticle composition (g dry weight/100 g)

	High amylose	4 g
	(50% amylose)	
25	Egg lecithin	2 g
	Alginic acid	2 g
	Aqua Savor (attractant)	2 g
	Concentration of bioactive ingredients	
	Primary composition	20 g
30	Secondary composition	2 g
	Water	100 mL

[0077] Example 2 -- Formulation of an antiviral combination

[0078] The primary composition contains polyphenols, preferably from Olea europa leaf extract (Naturex Inc., Mamaroneck, NY) standardized to 15% oleutropein. The secondary composition contains phenols, preferably from Origanum vulgare or cinnamon bark extract, standardized to 25% (w/w), or lapatcho extract from Tabebuia avellaneda.

5 [0079] Example 3 -- Antiviral preparation against shrimp viral diseases

[0080] Freeze-dried microparticles containing an antiviral combination are produced as described in Examples 1 and 2. The microparticulate composition is then included at 1% (w/w) in a shrimp standard grow-out diet. This formulation provides the shrimp with 200 parts per million of primary composition and 20 parts per million secondary composition enclosed in a protected system. The two bioactive agents act synergistically to increase bioavailability and absorption into the animal's blood stream.

15

[0081] Example 4 -- Feeding of shrimp (*Penaeus vannamei*) with an antiviral preparation

[0082] Shrimp fry at about 10 gram size are stocked at 10 kilograms per cubic meter of seawater at 28 degrees Celsius. Water quality is maintained by rapidly exchanging the tank water through mechanical and biofiltration systems. Shrimp are fed a pelleted diet as described in Example 3. The experiment is terminated when shrimp reach an average commercial size of 40 grams. Growth parameters are determined using the following formulas.

[0083] Growth Rate = (Final average weight minus initial average weight)/n days.

25 [0084] Food conversion ratio (FCR) = Total food given/(total final biomass minus total initial biomass).

[0085] A sample of 20 shrimp is placed in a contained tank and infected with white spot virus (WSV) and survival is recorded over 2 weeks. Another sample of 20 shrimp is also placed in a contained tank and infected with hepatopancreatic parvo-like virus (HPV) and 30 survival recorded over 2 weeks.

[0086] Example 5 -- Antiviral preparation against pathogenic viruses of fish

[0087] Droplets containing the antiviral preparation as described in Example 2 are moistened. Trout fry at about 100 gram size are stocked at 30 kilograms per cubic meter of freshwater at 15 degrees Celsius. Water quality is maintained by rapidly exchanging the tank water through mechanical and biofiltration systems. Fish are fed 4 times daily a total ration at 2% of body weight on a commercial feed and 10% (wet weight) of moistened droplets containing antiviral preparation as described in Example 2 for 21 days. Growth parameters are determined using the formulas in Example 4.

[0088] A sample of 20 fish is injected with Infectious Pancreatic Necrosis Virus (IPNV) in saline and placed in a contained tank. Survival is recorded over 2 weeks. Another sample of 20 fish is injected with hematopoietic Necrosis Virus (IHNV) containing saline and placed in a contained tank. Survival is recorded over 2 weeks period following the infection.

[0089] Example 6 -- Formulation of an antibacterial combination

[0090] The primary composition contains polyphenols, preferably from Vitis vinifera leaves extract (Naturex Inc., Mamaroneck, NY) standardized to 98% bioactive polyphenols. The secondary composition contains terpenes, preferably from Piper nigrum extract, standardized to 95% Piperin.

[0091] Example 7 -- Antibacterial preparation against pathogenic bacteria of marine fish larvae

[0092] Microparticles containing the antimicrobial formulation as prepared described in Examples 1 and 6 are moistened. The microparticles are sorted through fine mesh screens into 2 sizes: Size A microparticles contain small size particles of less than 10 micrometers and size B microparticles contain larger size particles of 50-100 micrometers. In general, size A microparticles are more suitable for feeding smaller organisms, and size B microparticles are more suitable for feeding larger organisms.

[0093] Example 8 -- Rotifer enrichment with an antibacterial preparation

[0094] Microparticles 10 millimeters in diameter containing an antibacterial formula are prepared as described in Examples 1 and 7. Rotifers are cultured in seawater or artificial

seawater at a salinity of 15-40 psu at 20-30 degrees Celsius. Rotifers are fed a feed such as AquaGrow Enhance (TM) (Advanced BioNutrition Corp., Columbia, MD). Up to 30% of the rotifers are harvested daily from the culture tank, rinsed with freshwater, then transferred to an enrichment tank at a concentration of 500,000 rotifers per liter. AquaGrow DHA, AA
5 and Enhance (TM) (Advanced BioNutrition Corp., Columbia, MD) are mixed with 10% of freeze-dried size A microparticles and rotifers are enriched with 0.05 grams per liter of the mixture at 4 hour intervals. Rotifers are harvested after 8-16 hours, rinsed with seawater and delivered to aquatic larvae.

10 [0095] Example 9 -- Artemia enrichment with an antibacterial preparation
[0096] Size A microparticles were produced as described in Example 7. Artemia cysts (Sanders Brine Shrimp Company, Layton, Utah) were hatched in seawater or artificially made seawater at a salinity of 15-40 psu and temperature between 25-30 degrees Celsius. Newly hatched Artemia nauplii Rotifers were harvested after 24 hours from the culture tank,
15 rinsed with fresh water and transferred to an enrichment tank at a concentration of 300,000 nauplii per liter. AquaGrow DHA (RTM), AquaGrow AA (RTM) and AquaGrow Enhance (RTM) (Advanced BioNutrition Corp., Columbia, MD) was mixed with 10% of freeze-dried size A microparticles and nauplii were enriched with 0.3 grams per liter of the mixture at 8 hour intervals. Nauplii were harvested after 16 hours, rinsed with seawater, and then
20 delivered to aquatic larvae. The antibacterial composition effected a significant improvement in the survival rate of the Artemia (Fig. 1).

[0097] Example 10 -- Feeding shrimp larvae with rotifers and Artemia loaded with an antibacterial preparation
25 [0098] Newly hatched Pacific white shrimp (*Litopenaeus vannamei*) are stocked at 100 larvae per liter of seawater. Larvae are fed four times daily with rotifers (at 5 rotifers per milliliter) loaded with antibacterial preparation as described in Examples 1 and 6. Larvae are also given a daily mixture of the live algae *Tetraselmis* sp. and *Chaetoceros* sp., at concentrations of 10,000 and 5,000 cells per milliliter, respectively. Zoea-1 larvae are
30 introduced with Artemia nauplii loaded with antibacterial preparation as described in Example 8. Four rations of Artemia at 10 nauplii per milliliters are given daily until larvae

have reached the post larval stage (PL1). Post larval shrimp are then counted in each tank to determine a survival rate and sampled for average weight.

[0099] A sample of 20 post larval shrimp are placed in a contained tank and infected with 1000 colony forming units per milliliter of *Pseudomonas aeruginosa*. Survival is

5 recorded over a 2 week period following the infection.

[0100] Example 11 -- Formulation of an antiparasitic combination

[0101] The primary composition contains phenols, preferably from *Trifolium pratense* leaf extract (Naturex Inc., Mamaroneck, NY), standardized to 25% phenols and glycosides.

10 The secondary composition contains alcohol(s), preferably from *Eucalyptus globulus* extract, standardized to 2% oil.

[0102] Example 12 -- Feeding post-larval sea bream with rotifers and Artemia loaded with an antiparasitic preparation

15 [0103] Rotifers and Artemia are loaded with an antiparasitic preparation as described in examples 1 and 11. Sea bream eggs are stocked in a larvae rearing system at a concentration of 100 eggs per liter and allowed to hatch in full seawater (32-40 ppt) at a temperature of 17-19 degrees Celsius. Larvae are commenced feeding 3 days post hatching with rotifers enriched with AQUAGROW DHA (RTM) and an antiparasitic preparation as

20 described in Example 11. Fourteen days post hatch, larvae are offered Artemia nauplii enriched with AQUAGROW DHA (RTM) and loaded with antiparasitic preparation as outlined in Example 9. Larvae are fed 3 times daily at a concentration of 10 nauplii per milliliter until day 36 post hatch. Tanks are then harvested, counted individually for survivorship, and sampled for average weight.

25 [0104] A sample of 20 post larval sea bream are placed in a contained tank and infected with 1000 cells per milliliter of Amyloodinium. Fish are fed Artemac-4 diet (Aquafauna Bio-Marine, Inc., Hawthorne, CA) supplemented with 10% of size B microparticles loaded with antiparasitic preparation as described in Example 11 and survival is recorded over a 4 week period following the infection.

[0105] Example 13 -- Formulation of an antifungal combination

[0106] The primary composition contains phenols, preferably from Curcuma longa extract (Naturex Inc., Mamaroneck, NY), standardized to 95% curcumin. The secondary composition contains phenols preferably from Rosmarinus officinalis extract standardized

5 to 2.5% oil.

[0107] Example 14 -- Feeding oysters with microparticles containing an antifungal preparation

[0108] Size A particles containing the antiparasitic preparation of Example 13 are prepared as described in Examples 1 and 13. Oysters, Crassostrea gigas, spats are stocked in a larvae rearing system at a concentration of 100 spates per liter in full seawater (32-40 ppt) at a temperature of 25-29 degrees Celsius. Oysters are given a daily mixture of the live algae Tetraselmis sp. and Chaetoceros sp. at concentrations of 10,000 and 5,000 cells per milliliter, respectively, with 5 milligrams per liter of freeze-dried antifungal preparation (as described in Examples 1 and 13) added until day 40 post hatch. Tanks are then harvested, counted individually for survivorship, and sampled for average weight.

[0109] A sample of 20 oysters is placed in a contained tank and infected with 1000 cells per milliliter of Marteilia refringens and fed with size A microparticles loaded with antifungal preparation as described in Examples 1 and 13. Survival is recorded over a 2 week period.

[0110] Example 15 -- Feeding cats with extruded feeds containing an antiviral preparation

[0111] A standard commercial cat feed is amended with 1% of the microparticle preparation from Examples 1 and 2. The mixture is then pelleted with an extruder and fed to 10 cats for 4 weeks. Cats are then injected with saline containing herpes virus. Conjunctivitis, keratoconjunctivitis (KCS), and discharge from the eye is monitored over a 3 week period.

[0112] Example 16 -- Feeding poultry with extruded feeds containing antiviral preparation

[0113] A standard commercial broiler feed is amended with 1% (w/w) of the microparticle preparation of Examples 1 and 2. The mixture is then pelleted with an extruder and fed to 10 broiler chickens at size of about 100 grams. Broilers are housed in windowless sheds at a stocking density of 20 kilograms of bird weight per cubic meter. Temperature and ventilation are automatically controlled. Broilers are fed 4 times daily a total ration of 4% body weight and pellet size adjusted to fit the mouth opening of the growing chick. The experiment is terminated after 4 weeks and the broilers are then 10 injected with saline containing chicken anemia agent (CAA) and mortality is monitored over a 3 week period.

[0114] Example 17 -- Feeding laying hens with beadlets containing an antibacterial preparation

15 [0115] Beadlets containing an antimicrobial preparation are prepared as described in Examples 1 and 6. The beadlets are then freeze-dried for later use.

[0116] Sixteen laying hens at size of about 500 grams are housed in windowless sheds at a stocking density of 20 kilograms of bird weight per cubic meter. Temperature and ventilation are automatically controlled. Broilers are fed a standard commercial diet 4 times 20 daily at a total ration of 4% body weight. Hens are also fed with 1% of daily ration with beadlets containing antibacterial preparation-II. The experiment is terminated after 4 weeks and hens are then injected with saline containing 100,000 Salmonella per milliliter. Morbidity and mortality are monitored over a 3 week period. Eggs are collected for a period of 4 weeks following infection and are monitored for Salmonella contamination.

25

[0117] Example 18 -- Feeding pigs with feed containing an antiviral preparation

[0118] A standard commercial swan feed is amended with 1% of microparticle antiviral preparation from Examples 1 and 2. The mixture is then pelleted with an extruder and fed to 10 pigs at age 3-5 weeks. Hogs are challenged with a Mycoplasma hyopneumoniae 30 virulent strain delivered via the intratracheal route on day 70, and lung lesion scores are then monitored for 21 days against an experimental challenge; treated and untreated hogs are compared.

[0119] References Cited

[0120] The following are incorporated by reference.

[0121] U.S. Patent Documents

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5 [0123] United States Patent 6,444,458 Singh, et al. September 3, 2002.

[0124] United States Patent 6,414,036 Ninkov July 2, 2002.

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I claim:

1. A stable, bioactive feed additive comprising a plant-derived bioactive primary composition and a plant-derived bioactive secondary composition, wherein the secondary composition is present at less than or equal to about 40% of the primary
5 composition.

2. A feed additive as in Claim 1, wherein the bioactive primary composition comprises a polyphenol, acid, phenol, or a combination thereof.

3. A feed additive as in Claim 2, wherein the primary composition comprises thymol, methyleugenol, acetyleugenol, safrol, eugenol, isoeugenol, anethole, phenol,
10 methylchavicol (estragol; 3-4-methoxyphenyl- 1-propene), carvacrol, alpha-bisabolol, fornesol, anisole (methoxybenzene), propenylguaethol (5-propenyl-2-ethoxyphenol), a tannin, an aromadendrine, an anthocyanin, a catecholin, a catechin, an oleuropein, a taxifolin, a plant oil, extracts from plants such as origanum, thyme, rosemary, orange, clove, fennel, camphor, mandarin, anise, cascarilla, estragon and pimento, or olive tree (leaf or
15 fruit extracts), green tea, coffee, cocoa, soya, green tea, onions, grapes, ginger, acetic acid, aconitic acid, adipic acid, formic acid, malic acid (1-hydroxysuccinic acid), capronic acid, hydrocinnamic acid (3-phenyl-1-propionic acid), pelargonic acid (nonanoic acid), lactic acid (2-hydroxypropionic acid), phenoxyacetic acid (glycolic acid phenyl ether), phenylacetic acid (alpha-toluenic acid), valeric acid (pentanoic acid), iso-valeric acid (3-methylbutyric
20 acid), cinnamic acid (3-phenylpropenoic acid), citric acid, mandelic acid (hydroxyphenylacetic acid), tartaric acid (2,3-dihydroxybutanedioic acid; 2,3-dihydroxysuccinic acid), fumaric acid, tannic acid, or a combination of two or more of these.

4. A feed additive as in claim 2, wherein the primary composition comprises at
25 least one volatile compound.

5. A feed additive as in Claim 4, wherein the primary composition comprises acetoin (acetyl methyl carbinol), ethyl alcohol (ethanol), propyl alcohol (1-propanol), iso-propyl alcohol (2-propanol, isopropanol), propylene glycol, glycerol, benzyl alcohol, n-butyl alcohol (n-propyl carbinol), iso-butyl alcohol (2-methyl-1-propanol), hexyl alcohol
30 (hexanol), L-menthol, octyl alcohol (n-octanol), cinnamyl alcohol (3-phenyl-2-propene-1-ol), alpha-methylbenzyl alcohol (1-phenylethanol), heptyl alcohol (heptanol), n-amyl alcohol (1-pentanol), iso-amyl alcohol (3-methyl-1-butanol), anisic alcohol (4-

methoxybenzyl alcohol, p-anisic alcohol), citronellol, n-decyl alcohol (n-decanol), geraniol, beta-gamma-hexenol (3-hexenol), lauryl alcohol (dodecanol), linalool, nerolidol, nonadieneol (2,6-nonadiene-1-ol), nonyl alcohol (nonanol-1), rhodinol, terpineol, borneol, clineol (eucalyptol), anisole, cuminaldehyde (cuminaldehyde), 10-undecene-1-ol 1-hexadecanol, 5 acetaldehyde, anisic aldehyde, benzaldehyde, iso-butyl aldehyde (methyl-1-propanal), citral, citronellal, n-capraldehyde (n-decanal), ethylvanillin, furfural, heliotropin (piperonal), heptyl aldehyde (heptanal), hexyl aldehyde (hexanal), 2-hexenal (beta-propylacrolein), hydrocinnamic aldehyde (3-phenyl-1-propanal), lauryl aldehyde (dodecanal), nonyl aldehyde (n-nonanal), octyl aldehyde (n-octanal), phenylacetaldehyde (1-oxo-2-phenylethane), propionaldehyde (propanal), vanillin, cinnamic aldehyde (3-phenylpropenal), perillaldehyde, cuminaldehyde, iso-amyl acetate (3-methyl-1-butyl acetate), benzyl acetate, benzylphenyl acetate, n-butyl acetate, cinnamyl acetate (3-phenylpropenyl acetate), citronellyl acetate, ethyl acetate (acetic ester), eugenol acetate (acetyleneugenol), geranyl acetate, hexyl acetate (hexanyl ethanoate), hydrocinnamyl acetate (3-phenylpropyl acetate), linalyl acetate, octyl acetate, phenylethyl acetate, terpinyl acetate, triacetin (glyceryl triacetate), potassium acetate, sodium acetate, sodium diacetate, calcium acetate, acetaldehyde dibutyl acetal, acetaldehyde dipropyl acetal, acetaldehyde phenethyl propyl acetal, cinnamic aldehyde ethylene glycol acetal, decanal dimethyl acetal, heptanal dimethyl acetal, heptanal glycetyl acetal, benzaldehyde propylene glycol acetal, allixin, 15 camphor, limonene, beta-caryophyllene, benzoquinones, naphthoquinones, anthraquinones, plastoquinone, ubiquinone, phytoquinone, lapacho, lapachones, beta lapachone, extracts from melissa, coriander, cardamon, eucalyptus, citriodora, cinnamon, lemon, lemon grass, melissa, citronella, lime, orange, lavender, mustard, onion, garlic, pepper, bitter orange, caraway, dill, lemon, peppermint, nutmeg apple, or a combination of two or more of these.

20 6. A feed additive as in Claim 1, wherein the bioactive secondary composition comprises a polyphenol, acid, phenol, or a combination thereof, and is present at less than or equal to about 40% of the primary composition.

7. A feed additive as in Claim 6, wherein the secondary composition comprises acetoin (acetyl methylcarbinol), ethyl alcohol (ethanol), propyl alcohol (1-propanol), iso-propyl alcohol (2-propanol, isopropanol), propylene glycol, glycerol, benzyl alcohol, n-butyl alcohol (n-propyl carbinol), iso-butyl alcohol (2-methyl-1-propanol), hexyl alcohol (hexanol), L-menthol, octyl alcohol (n-octanol), cinnamyl alcohol (3-phenyl-2-propene-1-

oil), alpha-methylbenzyl alcohol (1-phenylethanol), heptyl alcohol (heptanol), n-amyl alcohol (1-pentanol), iso-amyl alcohol (3-methyl-1-butanol), anisic alcohol (4-methoxybenzyl alcohol, p-anisic alcohol), citronellol, n-decyl alcohol (n-decanol), geraniol, beta-gamma-hexenol (3-hexenol), lauryl alcohol (dodecanol), linalool, nerolidol, 5 nonadieneol (2,6-nonadiene-1-ol), nonyl alcohol (nonanol-1), rhodinol, terpineol, borneol, clineol (eucalyptol), anisole, cuminalcohol (cuminal), 10-undecene-1-ol 1-hexadecanol, acetaldehyde, anisic aldehyde, benzaldehyde, iso-butyl aldehyde (methyl-1-propanal), citral, citronellal, n-capraldehyde (n-decanal), ethylvanillin, furfurol, heliotropin (piperonal), heptyl aldehyde (heptanal), hexyl aldehyde (hexanal), 2-hexenal (beta-propylacrolein), 10 hydrocinnamic aldehyde (3-phenyl-1-propanal), lauryl aldehyde (dodecanal), nonyl aldehyde (n-nonanal), octyl aldehyde (n-octanal), phenylacetaldehyde (1-oxo-2-phenylethane), propionaldehyde (propanal), vanillin, cinnamic aldehyde (3-phenylpropenal), perillaldehyde, cuminaldehyde, iso-amyl acetate (3-methyl-1-butyl acetate), benzyl acetate, benzylphenyl acetate, n-butyl acetate, cinnamyl acetate (3-15 phenylpropenyl acetate), citronellyl acetate, ethyl acetate (acetic ester), eugenol acetate (acetyleugenol), geranyl acetate, hexyl acetate (hexanyl ethanoate), hydrocinnamyl acetate (3-phenylpropyl acetate), linalyl acetate, octyl acetate, phenylethyl acetate, terpinyl acetate, triacetin (glyceryl triacetate), potassium acetate, sodium acetate, sodium diacetate, calcium acetate, acetaldehyde dibutyl acetal, acetaldehyde dipropyl acetal, acetaldehyde phenethyl 20 propyl acetal, cinnamic aldehyde ethylene glycol acetal, decanal dimethyl acetal, heptanal dimethyl acetal, heptanal glyceryl acetal, benzaldehyde propylene glycol acetal, allicin, camphor, limonene, beta-caryophyllene, benzoquinones, naphthoquinones, anthraquinones, plastoquinone, ubiquinone, phytoquinone, lapacho, lapachones, beta lapachone, extracts from melissa, coriander, cardamon, eucalyptus, citriodora, cinnamon, lemon, lemon grass, 25 melissa, citronella, lime, orange, lavender, mustard, onion, garlic, pepper, bitter orange, caraway, dill, lemon, peppermint, nutmeg apple, or a combination of two or more of these.

8. A feed additive as in claim 1, wherein the secondary composition comprises at least one volatile compound.

9. A feed additive as in Claim 8, wherein the secondary composition comprises 30 thymol, methyleugenol, acetyleugenol, safrol, eugenol, isoeugenol, anethole, phenol, methylchavicol (estragol; 3-4-methoxyphenyl- 1-propene), carvacrol, alpha-bisabolol, fornesol, anisole (methoxybenzene), propenylguaethol (5-propenyl-2-ethoxyphenol), a

tannin, an aromadendrine, an anthocyanin, a catecholin, a catechin, an oleuropein, a taxifolin, a plant oil, extracts from plants such as origanum, thyme, rosemary, orange, clove, fennel, camphor, mandarin, anise, cascarilla, estragon and pimento, or olive tree (leaf or fruit extracts), green tea, coffee, cocoa, soya, green tea, onions, grapes, ginger, acetic acid, 5 aconitic acid, adipic acid, formic acid, malic acid (1-hydroxysuccinic acid), capronic acid, hydrocinnamic acid (3-phenyl-1-propionic acid), pelargonic acid (nonanoic acid), lactic acid (2-hydroxypropionic acid), phenoxyacetic acid (glycolic acid phenyl ether), phenylacetic acid (alpha-toluenic acid), valeric acid (pentanoic acid), iso-valeric acid (3-methylbutyric acid), cinnamic acid (3-phenylpropenoic acid), citric acid, mandelic acid 10 (hydroxyphenylacetic acid), tartaric acid (2,3-dihydroxybutanedioic acid; 2,3-dihydroxysuccinic acid), fumaric acid, tannic acid, or a combination of two or more of these.

10. A feed additive as in Claims 1-9, wherein the ratio of said primary composition to said secondary composition ranges from about 10,000:1 to 10:4.

15 11. A feed additive as in Claims 1-9, wherein the ratio of said primary composition to said secondary composition ranges from about 1000:1 to 10:4.

12. A feed additive as in Claims 1-9, wherein the ratio of said primary composition to said secondary composition ranges from about 100:1 to 10:4.

13. A feed additive as in Claims 1-12, further comprising powdered lecithin, 20 cornstarch comprising 50% amylose, and at least one attractant.

14. A feed additive as in Claims 1-13, further comprising essential amino acids, essential fatty acids, sugars, polysaccharides, minerals, vitamins, proteases, lipases, amylases, or combinations of these.

15. A feed additive as in Claims 1-12, further comprising about 1% (w/v) 25 alginic acid, wherein the alginic acid is in the form of a cross-linked polymer.

16. A method for the protection against, prevention of, or cure of disease in an animal, wherein the method comprises providing a feed additive to the animal, said feed additive comprising a bioactive primary composition and a bioactive secondary composition in an amount sufficient to prevent, mitigate, or cure the disease, wherein the secondary 30 composition is present at less than or equal to about 40% of the primary composition.

17. A method as in Claim 16, wherein the animal is a terrestrial animal.

18. A method as in Claim 16, wherein the animal is an aquatic animal.

19. A method as in claim 16, wherein the bioactive primary composition and the bioactive secondary composition are as described in Claims 1-15.

20. The method as in Claim 19, wherein said additive is from about 1 ppm to 10% of the feed by weight.

5 21. The method as in Claim 19, wherein said additive is from about 0.001% to 0.5% of the feed by weight.

22. The method as in Claim 19, wherein said additive is from about 0.002% to 0.25% of the feed by weight.

23. The method for protection or prevention of disease in animals using any of
10 Claims 1-22.

24. The method as in claim 23 wherein the animals are terrestrial.

25. The method as in claim 23 wherein the animals are aquatic.

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Figure 1